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VOL. 23, SEC. C. AUGUST, 1945 NUMBER 4

THE EFFECT OF LEAF RUST ON THE YIELD AND QUALITY OF WHEAT[1]

BY B. PETURSON[2], MARGARET NEWTON[3], AND A. G. O. WHITESIDE[4]

Abstract

In field experiments carried out at Winnipeg, heavy artificially-induced infection of leaf rust of wheat reduced the yield, bushel weight, kernel weight, number of kernels per head, and percentage of flour yield of the varieties Thatcher, Apex, Renown, and Regent. The infection increased the yellow pigment content of the flour of all these four varieties but, apart from a reduction in flour yield, had no other adverse effect on the milling and baking quality of the grain. In fact, the flour milled from the rusted samples was superior in baking strength to the flour milled from the non-rusted samples. Under field conditions, the rust had a variable effect on the percentage of protein of the grain. In one year it increased the percentage of protein of the seed, but in two other years it decreased the percentage of protein. Under greenhouse conditions, in two different years, leaf rust on Thatcher wheat reduced the yield of seed, number of kernels per head, number of fertile tillers, and yield of straw, but increased the protein content of the seed, leaves, and straw.

Introduction

Field experiments carried out by two of the authors (9) in 1938 demonstrated that leaf rust (*Puccinia triticina* Erikss.) reduced the yield, quality, grade, and percentage protein of the two stem rust resistant varieties Thatcher and Renown. In 1940, these experiments were repeated and expanded to include two other resistant varieties, namely, Regent and Apex. As the results obtained in 1940 were not in accord with those of 1938 in respect to the effect of leaf rust on the percentage of protein of the seed, the experiments were continued in 1941 and 1943. In 1941, only the varieties Thatcher and Renown were tested, but, in the experiment of 1943, the varieties tested were the same as those included in the 1940 test. During 1940 and 1941, two greenhouse experiments were also conducted to determine the effect of leaf rust, initiated at three different stages of host development, on the yield and protein content of wheat. The present paper gives the results obtained in both field and greenhouse experiments.

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Field Tests

Materials and Methods

The field experiments consisted of 16 plots of each variety in 1940, 20 in 1941, and 12 in 1943. Each plot consisted of three rod rows. Half of the plots of each variety were kept as free as possible from rust infection by dusting them with sulphur (Kolodust) at the rate of 30 lb. per acre per dusting. Applications of dust were made three times a week as well as after every heavy rain from the time the plants reached the five-leaf stage until about a week before the flag leaves withered. The remaining plots were inoculated with a mixture of some 30 different physiologic races of leaf rust when the plants had reached the five-leaf stage. At maturity, the centre row of each plot was harvested to obtain yield and quality data, and 100 heads were taken at random from the side rows of each plot to determine the number of kernels per head.

The percentages of leaf rust were estimated while the leaves were still green. The estimates were based on the scale of stem rust percentages adopted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

For each plot in the 1940 and 1943 field tests, the yield, number of kernels per head, bushel weight, and 1000-kernel weight were determined and, for each plot in the 1941 field test, the yield, bushel weight, and 1000-kernel weight were taken. The bulked samples of grain from the check plots and from the rusted plots in the tests of 1940 and 1943 were each tested for milling and baking quality, and the grain from the check and rusted plots in all three field tests was analysed for protein content.

The milling and baking tests were carried out in the Milling and Baking Laboratory of the Cereal Division, Experimental Farms Service, Ottawa, and the protein analyses in the Chemical Laboratories, Science Service, Ottawa.

Experimental Results

Severity of Infection in the Plots

In 1940, all the infected plots of the two susceptible varieties, Thatcher and Apex, became quite heavily rusted, those of the two moderately resistant ones, Regent and Renown, rusted but lightly. In 1941, the infected plots of Thatcher again rusted heavily, while only light rust infection developed on the infected plots of Renown. All the infected plots of all four varieties became very heavily rusted in 1943. In that year, Regent and Renown rusted almost as heavily as Thatcher and Apex. Apparently the rust infection became firmly established on Regent and Renown before the adult plant resistance to leaf rust, a characteristic of these two varieties, became operative. In the check plots in all three years, control of rust was very satisfactory—traces of rust occurring in 1940 and 1941 and only very light infections in 1943.

Effect on Yield, Bushel and Kernel Weight, and Number of Kernels per Head

As shown in Table I, heavy infection of leaf rust very materially reduced the yield of seed of all four varieties tested. Light infection depressed the the yield of seed but proportionately less than did heavy infection. The bushel and kernel weights of the varieties likewise were adversely affected by leaf rust, reductions being quite substantial from heavy infection and measurable even from moderately light infection. Leaf rust generally caused slight reductions in the number of kernels per head. In fact, except in the variety Thatcher in the test of 1943, the rusted plants in every test produced slightly fewer kernels per head than the corresponding check plants.

The analysis of variance results showed that—with the exception of the difference in bushel weight of Regent in 1940 and the differences of yield and bushel weight of Renown in 1941—the reductions in yield, in bushel weight, and in kernel weight were statistically significant. However, none of the differences in number of kernels per head between comparable rusted and check plants were significant. The data for the characters concerned for all three field experiments are given in Table I.

Effect of Leaf Rust on Protein Content

Leaf rust slightly increased the percentage of protein of the seed of all four varieties in 1940. The percentage increase in protein was greater in the heavily rusted varieties Thatcher and Apex than in the lightly rusted ones Renown and Regent. However, contrary results were obtained in 1941 and 1943. In both these years, leaf rust decreased the percentage of protein of the varieties tested. The reductions in percentage of protein in 1941 were statistically significant for both Thatcher and Renown, the two varieties tested in that year. In 1943, the reductions were again significant for Thatcher and Renown, but were slightly below the level of significance for Regent and definitely not significant for Apex. With respect to the effect of leaf rust on percentage of protein, the results of the experiments carried out in 1941 and 1943 were entirely in accord with field tests made in 1938 (9). Although these tests strongly indicate that leaf rust generally reduces the percentage of protein of the seed of wheat varieties, it evidently may, under certain conditions, actually increase the percentage of protein of the seed, as occurred in 1940. It is not at all clear, at present, why leaf rust increased the percentage of protein of the seed in one year and decreased it in the other years.

Effect of Leaf Rust on Milling and Baking Quality

The results of the milling and baking tests (Table II) show that, in both 1940 and 1943, leaf rust slightly reduced the flour yield of Thatcher, Apex, Renown, and Regent, and increased the yellow pigment content of their flour. These results are in agreement with those obtained in 1938. On the other hand, the milling and baking quality of the grain was not adversely affected, except in respect to flour yield and yellow pigment content. Actually, the flour milled from the rusted samples, except those of Renown in 1940, was

TABLE I

THE EFFECT OF LEAF RUST ON THE YIELD, WEIGHT PER BUSHEL, KERNEL WEIGHT, AND NUMBER OF KERNELS PER HEAD OF THATCHER, APEX, RENOWN, AND REGENT WHEAT GROWN IN FIELD PLOTS IN 1940, 1941, AND 1943

Variety	Treatment	Amount of infection	Average yield per acre, bu.	Decrease in yield, %	Average weight per bushel, lb.	Average weight per 1000 kernels, gm.	Decrease in weight per 1000 kernels, %	Average number of kernels per head	Decrease in number of kernels per head, %
Experiment of 1940									
Thatcher	Check	Trace	46.18		62.0	26.28		26.28	
Thatcher	Rusted	90%	20.16	56.3	54.2	15.57	40.7	24.66	4.5
Apex	Check	Trace	33.27		62.9	31.76		20.35	
Apex	Rusted	75%	18.87	43.3	58.3	22.27	29.9	20.08	1.3
Renown	Check	Trace	37.69		63.4	29.08		23.68	
Renown	Rusted	15%	29.13	22.7	62.2	25.29	13.0	23.01	2.8
Regent	Check	Trace	37.21		61.3	30.87		23.48	
Regent	Rusted	15%	31.19	16.1	60.8	27.70	10.3	23.30	0
Necessary difference*			3.1		1.2	1.1		**	
Experiment of 1941									
Thatcher	Check	Trace	45.54		63.5	26.88		—	—
Thatcher	Rusted	70%	36.34	20.1	62.0	24.16	10.1	—	—
Renown	Check	Trace	35.26		64.3	30.80		—	—
Renown	Rusted	14%	32.81	6.9	64.1	29.75	3.4	—	—
Necessary difference*			2.9		0.7	1.0			
Experiment of 1943									
Thatcher	Check	10.0%	45.40		64.2	31.90		25.05	+1.02
Thatcher	Rusted	83.0%	27.10	40.3	60.1	23.16	27.4	26.07	
Apex	Check	10.0%	23.90		63.8	37.06		14.64	
Apex	Rusted	77.0%	16.50	31.0	61.4	29.02	21.7	12.23	16.5
Renown	Check	10.0%	44.60		65.3	36.86		18.61	
Renown	Rusted	70.0%	19.70	55.8	60.8	26.04	29.4	17.32	6.9
Regent	Check	10.0%	35.30		63.2	39.68		17.35	
Regent	Rusted	71.0%	21.50	39.1	59.3	28.76	27.5	16.78	3.3
Necessary difference*			7.4		1.1	2.3		**	

* *Necessary difference, 5% level of significance, between means of rusted and check plots within a given variety.*

** *Difference between the rusted and check plots insignificant.*

TABLE II

THE EFFECT OF LEAF RUST ON THE MILLING AND BAKING QUALITY OF THATCHER, APEX, RENOWN, AND REGENT WHEAT GROWN IN FIELD PLOTS IN 1940, 1941, AND 1943

(Malt–phosphate–bromate baking method)

Variety	Treatment	Amount of infection	Wheat protein, %	Flour yield, %	Flour colour (yellow pigment), p.p.m.	Loaf volume, cc.	Crumb[1] texture	Crumb[1] colour
Experiment of 1940								
Thatcher	Check	Trace	15.6	71.8	1.8	848	8.0	7.0
Thatcher	Rusted	90%	16.2	70.1	2.6	963	7.8	5.5
Apex	Check	Trace	15.3	72.1	1.6	820	8.0	8.3
Apex	Rusted	75%	16.1	70.7	2.0	938	8.0	7.5
Renown	Check	Trace	16.2	75.2	1.8	880	7.5	7.0
Renown	Rusted	15%	16.4	74.1	2.0	935	7.5	6.8
Regent	Check	Trace	16.8	74.9	1.6	980	7.8	8.3
Regent	Rusted	15%	17.1	74.8	1.9	993	8.0	8.0
Experiment of 1941								
Thatcher	Check	Trace	15.8	—	—	—	—	—
Thatcher	Rusted	70%	14.3	—	—	—	—	—
Renown	Check	Trace	16.3	—	—	—	—	—
Renown	Rusted	14%	15.7	—	—	—	—	—
Necessary difference*			0.47					
Experiment of 1943								
Thatcher	Check	10%	15.3	76.1	1.7	827	7.8	7.8
Thatcher	Rusted	83%	14.0	75.6	2.5	872	7.8	6.5
Apex	Check	10%	16.2	75.1	1.3	855	7.8	8.7
Apex	Rusted	77%	15.7	73.5	1.8	877	8.0	7.5
Renown	Check	10%	15.0	76.9	1.6	868	8.0	7.3
Renown	Rusted	70%	14.1	76.3	2.4	845	8.0	6.8
Regent	Check	10%	15.6	77.5	1.5	835	8.2	7.8
Regent	Rusted	71%	15.3	75.7	2.1	935	8.0	6.7
Necessary difference*			0.76					

[1] *Maximum scores: crumb texture, 10; crumb colour, 10.*

* *Necessary difference, 5% level of significance, between means of rusted and check plots within a given variety.*

slightly superior in baking strength (as indicated by loaf volume) to the flour milled from the non-rusted samples. These results are entirely in accord with those of Greaney *et al.* (3), who found that baking strength in Marquis wheat, as judged by loaf volume and crumb texture, was not seriously affected by stem rust.

Greenhouse Tests

Materials and Methods

In two greenhouse experiments Thatcher wheat was inoculated at different stages of maturity with race 76 of leaf rust to ascertain the effect of this rust on wheat when infections were initiated at different stages of host development. One of these experiments was carried out in 1940 and the other in 1941. The plants were grown in 7-in. pots, with four plants in each pot. There were 60 pots in the first experiment and 40 in the second. In each experiment, the pots were divided into four equal groups. One group was infected when the plants had reached the seven-leaf stage, a second group when the plants had reached the heading stage, and a third when the plants had reached the flowering stage. The fourth group was not inoculated and served as a check.

In both tests, all the infected plants became quite severely rusted while the check plants remained almost entirely free of rust. The following data were taken for each pot: weight of seed, weight of straw, weight per 1000 kernels, number of kernels per head, and height of plant. In addition, for each treatment, the samples of grain, straw, and leaves were respectively bulked, and analysed for protein content. The grain from the main heads and from the tiller heads were analysed separately.

Effect on Yield, Kernel Weight, Number of Kernels per Head, Tiller Survival, and Plant Height

Leaf rust significantly reduced the number of kernels per head, the kernel weight, and the yield of seed of all infected groups of plants in both experiments. The weight of straw, except in the group infected at the flowering stage in the test of 1940, was also significantly reduced. The greatest reductions in yield and in number of kernels occurred in the plants infected at the seven-leaf stage and the least in those infected at the flowering stage. Contrary to expectations, the plants infected at the seven-leaf stage produced plumper and heavier kernels than those infected at the flowering stage, but much fewer kernels per plant. It would seem that the reduction in number of kernels in the former plants increased the proportion of food available per kernel for the remaining kernels, thus causing them to be heavier and plumper.

Owing to the fact that, in each group of plants, all tillers in excess of two per plant were removed as they appeared, it was not possible to estimate accurately the effect of leaf rust on tillering. However, by noting at time of harvest the mortality among the tillers that were permitted to develop, some idea of the effect of leaf rust on tiller survival was ascertained. The tillers in the groups infected at the seven-leaf and heading stages in the test of 1940 and those in all three of the infected groups in the test of 1941 were signifi-

cantly reduced by leaf rust, the mortality rate in the plants infected earliest being particularly heavy. No adverse effect on tiller survival in the group infected at the flowering stage in the test of 1940 was observed.

Leaf rust significantly reduced the height of the plants infected at the seven-leaf stage in both tests, but had no apparent effect on the height of those infected at the heading and flowering stages.

Most of the loss in yield in the plants infected at the seven-leaf stage was due to a reduction in the number of kernels, only a small proportion of it being due to a reduction in kernel weight. On the other hand, in the plants infected at the flowering stage, the loss in yield was chiefly due to a reduction in kernel weight and only secondarily to a reduction in the number of kernels. The results of the greenhouse tests, in respect to the effect of leaf rust on the yield of straw, height of plant, and kernel production agree with those of Johnston and Miller (5) in that all these characters were adversely affected by this rust. The relevant data are given in Table III.

TABLE III

THE EFFECT OF LEAF RUST ON THE YIELD OF SEED AND STRAW, WEIGHT PER 1000 KERNELS, AND ON THE REDUCTION OF TILLERS OF THATCHER WHEAT INOCULATED AT THE SEVEN-LEAF, HEADING, AND FLOWERING STAGE IN THE GREENHOUSE

Treatment	Amount of infection	Average yield of seed per pot, gm.	Reduction in yield, %	Average weight per 1000 kernels, gm.	Reduction in kernel weight, %	Average number of kernels per plant	Reduction in kernels, %	Average yield of straw per pot, gm.	Reduction in yield of straw, %	Reduction in tillers, %
Experiment of 1940										
Uninoculated	Trace	12.14	—	36.7	—	82.90	—	13.68	—	—
Inoculated at 7-leaf stage	85%	5.37	55.7	33.1	9.8	40.60	51.1	8.14	40.5	28.0
Inoculated at heading stage	75%	8.17	32.7	34.5	5.8	61.10	26.3	11.67	14.7	8.0
Inoculated at flowering stage	75%	10.21	15.9	32.2	12.2	79.50	4.1	13.07	4.4	0.0
Necessary difference*		1.35		1.37		6.88		0.97		
Experiment of 1941										
Uninoculated	Trace	5.70	—	26.6	—	53.50	—	3.80		—
Inoculated at 7-leaf stage	75%	1.91	66.5	22.9	13.6	20.97	45.9	1.75	53.9	42.8
Inoculated at heading stage	95%	2.07	63.7	16.3	38.6	32.10	32.8	2.67	29.7	20.8
Inoculated at flowering stage	85%	2.55	55.3	18.9	28.8	34.10	25.7	3.38		24.7
Necessary difference*		0.37		2.35		3.68		0.56		

* *Necessary difference, 5% level of significance, between treatment means.*

Effect on the Protein Content of Seed, Leaves, and Straw

Table IV gives the percentage of protein of seed, leaves, and straw of each group. This table shows that, in these greenhouse experiments, leaf rust generally increased the protein content of all plant parts analysed. The

TABLE IV

THE EFFECT OF LEAF RUST ON THE PROTEIN CONTENT OF GRAIN, LEAVES, AND CULMS OF THATCHER WHEAT INOCULATED AT THE SEVEN-LEAF, HEADING, AND FLOWERING STAGE IN THE GREENHOUSE

Treatment	Amount of infection	Protein			
		(N × 5.7)		(N × 6.25)	
		Grain			
		Main heads, %	Tiller heads, %	Leaves, %	Culms, %
Experiment of 1940					
Uninoculated	Trace	13.04	13.70	3.31	1.23
Inoculated at 7-leaf stage	80%	14.23	15.15	10.28	2.25
Inoculated at heading stage	75%	13.23	13.65	9.05	1.75
Inoculated at flowering stage	75%	13.27	13.48	7.74	1.35
Experiment of 1941					
Uninoculated	Trace	17.30	18.80	12.81	6.98
Inoculated at 7-leaf stage	70%	16.70	19.05	17.56	9.35
Inoculated at heading stage	95%	20.05	21.50	17.92	9.85
Inoculated at flowering stage	85%	19.30	20.40	18.24	8.19

increase in percentage of protein of the leaves was very pronounced, that of the straw and seed less so. The protein content of the seed from the tiller heads was higher than that of the main heads, and, in general, the heavier the rust infection that a group of plants carried, the higher was the protein content of its straw, leaves, and seed. In respect to the effect of leaf rust on protein content of leaves and straw, the results are in agreement with those of Caldwell *et al.* (2), who showed that leaf rust increased the protein content of straw and leaves of wheat, and with those of Shutt (10), Snyder (11, pp. 228-231), and Greaney *et al.* (3), all of whom found that rusted straw contained a greater percentage of protein than plants bearing little or no rust.

Discussion

These experiments clearly demonstrate that leaf rust very adversely affects susceptible varieties and that, under certain environmental conditions, such as prevailed in the test plot in 1943, varieties moderately resistant to leaf rust, such as Regent and Renown, may be severely injured by this rust. These two varieties possess, under ordinary weather conditions, a considerable degree of mature plant resistance to leaf rust, but may, under certain conditions, fail to develop this type of resistance early enough in the season to prevent heavy infection.

With respect to the effect of leaf rust on the protein content of leaves and straw of wheat, the results of the present study are very definite and clearly show that the leaves and straw of leaf-rusted wheat plants are materially higher in protein content than are the leaves and straw of comparable non-rusted plants. A careful literature survey has failed to reveal any contrary results.

That conflicting results should be obtained in this study with respect to the effect of leaf rust on the percentage of protein of grain is not altogether surprising, as the literature dealing with the effect of different rusts on the protein content of grain contains contradictory evidence. Broadfoot (1) found, in experiments conducted in 1927, that grain samples from plots heavily infected with leaf rust were slightly higher in protein content than samples from corresponding non-rusted plots. In greenhouse tests carried out by Murphy (8) in 1935, crown rust increased the protein content of oat kernels. The present authors (9) found, in a field test carried out in 1938, that leaf rust decreased the protein content of grain, a result in agreement with those obtained by Caldwell *et al.* (2) in 1930 and Waldron (12), in 1935, who found that leaf rust significantly reduced the protein content of grain samples of susceptible wheat varieties. Shutt (10) and Snyder (11), who analysed badly rusted samples of wheat and samples comparatively free from rust in 1904, found that seed of the badly rusted plants was about 3% higher in protein than seed of the relatively rust-free plants. On the other hand, Greaney *et al.* (3), Headden (4, pp. 97-99), and Mangels and Sanderson (7) found that stem rust significantly reduced the protein content of the grain of wheat varieties.

Clearly, then, the relationship between rust infection and percentage of protein is not a simple one. Under certain environmental conditions, it would appear that leaf rust increases the percentage of protein laid down in the kernels, while, under other conditions, it decreases it, but what combination of interacting factors, such as temperature, humidity, precipitation, sunlight, etc., causes leaf rust to affect protein assimilation adversely in some seasons and favourably in others is not known at present.

It may be concluded, from the milling and baking tests, that leaf rust adversely affects the yellow pigment content of the flour milled from rusted wheat and somewhat reduces the yield of flour, but does not impair its baking

strength. These results, in general, are in accord with the results obtained by Greaney *et al.* (3) on the effect of stem rust on the milling and baking properties of wheat, except that with stem rust the effects were more pronounced. A heavy infection of stem rust (80 to 90%) decreased the yield of flour by about 10% and increased the yellow pigment content of the flour by about 1.70 p. p. m., whereas, in the present study, a 90% infection of leaf rust reduced the flour yield by only about 2% and increased the yellow pigment content of the flour by about 0.60 p. p. m.

The field test results reported here agree with the results obtained by Waldron (12), namely, that, under field conditions, loss in yield from leaf rust is chiefly caused by a reduction in kernel weight and only secondarily by a reduction in the number of kernels. However, Caldwell *et al.* (2) found that leaf rust reduced wheat yields in the field chiefly by reducing the number of kernels. The greenhouse results confirm the work of Mains (6), who found in greenhouse experiments that, when infections occur late in the development of the plants, leaf rust causes a reduction in yield chiefly by reducing the weight of the individual kernel, and that, when infections occur at an early stage, the reduction in yield is due largely to reduction in the number of kernels per head. According to the greenhouse results obtained by Mains (6) and the authors, the discrepancies between the field results obtained by Waldron (12) and the authors and those obtained by Caldwell *et al.* (2), respecting the manner in which leaf rust causes yield reduction, appear to be due to the fact that the stage of development reached by the plants before rust becomes severe determines whether reduced kernel production or reduced kernel weight is responsible for the major reduction in yield.

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RESPONSES OF PLANT STEM CUTTINGS TREATED WITH CHEMICALS IN A CARRIER DUST[1]

By N. H. Grace[2]

Abstract

Plant stem cuttings were treated in a factorial series with talc dusts containing naphthylbutyric acid, nutrient salts, an organic mercurial disinfectant, finely divided silver metal, thiamin, and nicotinic acid. Naphthylbutyric acid treatment increased the number of roots for cuttings of *Physocarpus* and *Iresine* and reduced the mean root length of *Lonicera*. Nutrient salt treatment increased the number of roots on *Iresine* cuttings and the mean root length of *Lonicera*. Differential effects were found for treatments with naphthylbutyric acid and each of organic mercury, metallic silver, and nutrient salts. Organic mercury at 50 p.p.m. and metallic silver at 5000 p.p.m. were similar in their effects. Thiamin and nicotinic acid had no significant effect on the rooting responses.

Naphthylacetic acid was prepared in talc at concentrations of 4000, 2000, 1000, and 500 p.p.m. and indolylbutyric, naphthylbutyric acids, and potassium naphthylhexoate at molecularly equivalent concentrations. When applied in a talc carrier dust to cuttings of seven species naphthylacetic and indolylbutyric acids were substantially similar in their effects while naphthylbutyric acid and potassium naphthylhexoate showed a lower order of physiological activity. Effects of chemicals judged by the rooting responses of cuttings tended to be similar over the concentration range. On the average, the 4000 p.p.m. dosage was injurious, while the two lowest dosages failed to differ in effects between themselves.

Introduction

The rooting responses of plant cuttings have been shown to be affected by treatment with talc dusts containing cane sugar, nutrient salts, disinfectant, and phytohormone chemicals (8, 10, 13, 14, 16, 17, 18). This communication includes a further report on use of similar dusts with preliminary results on the use of thiamin and nicotinic acid. Finely divided silver metal in talc also was tested for effect on rooting of cuttings since exceedingly low concentrations are known to possess bacteriostatic activity (3, 4), and a comparison with fungicidal organic mercurials was desired.

Acids of the homologous naphthyl and indolyl series have been shown to affect the rooting responses of plant cuttings (6, 7, 9, 21). The earlier investigations of the physiological activity of these and related chemicals have involved use of solutions for treatment. The carrier dust method of applying synthetic stimulants to cuttings has also been shown to be effective (5, 8, 11 to 14, 22). The order of effectiveness of chemicals may vary with the method of application, and it is desirable that comparison be made by the dust method of treatment. A mixture of 1- and 2-γ-naphthylbutyric acids in a talc carrier has shown greater physiological activity than the corresponding mixture of potassium salts. Conversely, the salt of Σ-(1-naphthyl)-hexoic acid was the more active (11). While the mixture of naphthylbutyric acids has been

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TABLE I

SPECIES, CHEMICAL TREATMENTS, AND DATES OF PLANTING AND REMOVING CUTTINGS

Species	Factorial treatments (concentration of chemical in talc)					Groups of cuttings outside main factorial series (treatments in a talc carrier)	Number of:		Date	
	Naphthylbutyric acid, p.p.m.	Nutrient salts*, %	Organic mercury, p.p.m.	Vitamin, p.p.m.	No. of groups in factorial series		Cuttings per group	Replicates	Planted	Removed
Physocarpus opulifolius Maxim.	0 2000	0 1 10	0 50		12	Untreated 5000 p.p.m. metallic silver 5000 p.p.m. metallic silver and 2000 p.p.m. naphthylbutyric acid	9	3	13-2-41	31-3-41
Physocarpus opulifolius Maxim.	0 1000	0 1		0 100 (thiamin)	8	Untreated 50 p.p.m. of each of thiamin and nicotinic acid 1000 p.p.m. thiamin	8	3	13-2-41	1-4 41
Lonicera tatarica L.	0 1000	0 1		0 {50 (thiamin) {50 (nicotinic acid) 100 (thiamin)	12	Untreated 1000 p.p.m. thiamin 1000 p.p.m. naphthylbutyric acid + 100 p.p m. thiamin 8 days after planting 1000 p.p.m. naphthylbutyric acid + 100 p.p.m. thiamin 14 days after planting Untreated cuttings, 1000 naphthylbutyric acid applied 8 days after planting	10	3	15-1-41	10-2-41
Iresine Lindeni Lem.	0 1000	0 1		0 {50 (thiamin) {50 (nicotinic acid) 100 (thiamin)	12	Untreated	10	3	8-10-40	19-11-40

* *Hoagland's mixture of nutrient salts (14, 17, 19, 20).*

applied to cuttings of a number of species (12, 14, 17), potassium naphthylhexoate has not received as complete study (11). This communication describes experiments in which the physiological activity of the mixture of isomeric naphthylbutyric acids (hereafter designated naphthylbutyric acid) and potassium naphthylhexoate were compared with that of the more widely used 1-naphthylacetic and γ-(3-indolyl)-butyric acids.

Part I. Responses of Plant Cuttings to Treatment with Talc Dusts Containing Phytohormone, Nutrient Salts, Disinfectant Chemicals, Thiamin, and Nicotinic Acids

Cuttings of three plant species were treated with talc dusts prepared in the manner previously described (5, 8, 17). The species, dusts, and other treatments used, together with dates of planting in sand and removing cuttings are listed in Table I. Experiments were replicated and factorial and non-factorial treatment groups were inter-randomized. When cuttings were removed, record was taken of the number callused, rooted, rooted and with new top growth, and not rooted with new growth. The number of roots and their lengths were determined, and fresh root and green new growth weights also were taken. All data were subjected to analyses of variance, the numbers of cuttings being first transformed to angles (1).

Results

Effects of Phytohormone, Nutrient Salts, and Disinfectant Chemicals

Differential effects of naphthylbutyric acid, nutrient, and disinfectant chemicals on responses of *Physocarpus* cuttings are described by the data of Table II. Increasing concentrations of nutrient salts tended to reduce root

TABLE II

DIFFERENTIAL EFFECTS OF NAPHTHYLBUTYRIC ACID, NUTRIENT SALTS, ORGANIC MERCURY, AND METALLIC SILVER ON ROOTING RESPONSES OF *Physocarpus* CUTTINGS

	Naphthylbutyric acid in talc, p.p.m.					
	0	2000	0	2000	0	2000
	No. of roots per cutting		Fresh root weight per cutting, gm.		Fresh weight of new growth per cutting, gm.	
Nutrient in talc, %						
0	5.3	5.4[1]	0.11	0.09[1]		
1	4.0	4.8	0.10	0.09		
10	3.6	7.5	0.06	0.17		
Organic mercury in talc, p.p.m.						
0	5.0	5.2[2]	0.12	0.11[2]		
50	2.5	6.6	0.06	0.13		
Metallic silver in talc, p.p.m.						
0	7.0	4.5[3]			0.56	0.68[3]
5000	5.2	7.6			0.74	0.49

[1] *Averages of six observations.*
[2] *Averages of nine observations.*
[3] *Averages of three observations.*

number and fresh weight. Naphthylbutyric acid alone had little effect, but, when applied together with the 10% nutrient salt level, increased both the number of roots and the fresh root weight. Organic mercury treatment tended to reduce root number and fresh weight but when used with naphthylbutyric acid produced mildly beneficial results. The nature of the differential effects for metallic silver and naphthylbutyric acid on root numbers was substantially similar.

Metallic silver and naphthylbutyric acid effected increase of fresh weight of new green growth when each chemical was used separately; when used in conjunction, however, weight was reduced. These differential effects are substantially similar to those already mentioned for silver and naphthylbutyric acid on root number since weight of green growth tends to vary inversely with number or weight of roots (9).

Effects of Phytohormone, Nutrient Salts, Thiamin, and Nicotinic Acid

The fresh root weight of *Physocarpus* cuttings was increased on the average from 46 mgm. in the absence of naphthylbutyric acid to 94 mgm. per cutting following its application at the 1000 p.p.m. level in talc.

Differential effects of naphthylbutyric acid and nutrient salts on the mean root length of *Lonicera* are described in Table III. Nutrient treatment increased root length, naphthylbutyric acid had little effect, while use of these chemicals in conjunction led to reduced root length.

TABLE III

DIFFERENTIAL EFFECTS OF NAPHTHYLBUTYRIC ACID AND NUTRIENT SALTS ON MEAN ROOT LENGTH (MM.) OF *Lonicera* CUTTINGS

	Naphthylbutyric acid in talc, p.p.m.	
	0	1000
	Mean root length, mm.	
Nutrient in talc, %		
0	12.8	12.4
1	14.2	9.0
Mean	13.5	10.7

The effects of the treatments outside the main factorial series on responses of *Lonicera* cuttings are given in Table IV. Use of 1000 p.p.m. naphthylbutyric acid and 100 p.p.m. thiamin at time of planting yielded more and longer roots than occurred when application of the phytohormone at time of planting was followed by treatment with 100 p.p.m. thiamin 8 and 14 days later. (Results are averages for 8 and 14 day treatments.) It is also shown that application of the phytohormone in the usual manner, i.e. at time of planting, resulted in 97% rooted cuttings, whereas when cuttings were planted

TABLE IV

EFFECTS OF TREATMENT ON LENGTH AND NUMBER OF ROOTS AND NUMBER OF ROOTED *Lonicera* CUTTINGS

Dust treatment	No. of roots per cutting	Length of roots per cutting, mm.	Rooted cuttings	
			Transformed data	%
Naphthylbutyric acid (1000 p.p.m.) + 100 p.p.m. thiamin	13.1	172		
Naphthylbutyric acid (1000 p.p.m.) and 100 p.p.m. thiamin added 8 and 14 days after planting*	7.1	75		
Necessary difference, 5% level	3.6	82		
Naphthylbutyric acid (before planting), 1000 p.p.m.			83.9	97
Naphthylbutyric acid (8 days after planting), 1000 p.p.m.*			57.7	70
Necessary difference, 5% level			20.5	

* *Cuttings removed from medium, dusted, and replanted.*

without treatment, removed in eight days, and then dusted with the phytohormone, only 70% rooting was attained.

The effects of treatment on the number of roots per rooted *Iresine* cutting are given in Table V. Naphthylbutyric acid and nutrient salt treatments resulted, on the average, in an increase in the number of roots per rooted cutting. Untreated cuttings and those treated with talc alone had substantially equal numbers of roots.

TABLE V

AVERAGE EFFECTS OF TALC, NAPHTHYLBUTYRIC ACID, AND NUTRIENT SALTS ON ROOTING OF *Iresine* CUTTINGS

Treatment	Concentration of chemicals	No. of roots per cutting
Untreated		8.3
Talc alone		8.5
Average of all talc		11.9
Naphthylbutyric acid in talc, p.p.m.	0	10.0
Naphthylbutyric acid in talc, p.p.m.	1000	13.7
Nutrient salts in talc, %	0	10.6
Nutrient salts in talc, %	1	13.2

Discussion

These effects of phytohormone, nutrient salts, and an organic mercurial disinfectant are in general agreement with the results of earlier experiments (8, 10, 13, 14, 16, 17, 18). Metallic silver and organic mercury in a talc carrier dust affect certain responses similarly. It would be of interest to investigate the effects of finely divided metallic silver in the treatment of cuttings markedly subject to bacterial infections.

Since thiamin and nicotinic acid treatments had no over-all effects on any of the rooting responses, use of these chemicals in dusts for the treatment of plant stem cuttings seems unlikely. The suggested favourable effect from use of 100 p.p.m. thiamin along with phytohormone at time of planting rather than 8 or 14 days after phytohormone treatment (Table IV) may relate to moving the cuttings before roots were manifested. The beneficial effect of phytohormone treatment at time of planting rather than eight days later when callused cuttings were removed for treatment is of interest but this finding may also relate to injury from handling.

Part 2. Rooting Responses of Cuttings to Treatment with Naphthyl and Indolyl Derivatives in a Talc Carrier

The chemicals were mixed with a talc carrier in the manner previously described (5, 8). Naphthylacetic acid dusts were prepared at concentrations of 4000, 2000, 1000, and 500 p.p.m. (parts of chemical per million parts of talc mixture by weight). Naphthylbutyric acid, potassium naphthylhexoate, and indolylbutyric acid were each prepared separately in talc at four concentrations molecularly equivalent to those containing naphthylacetic acid. Thus the investigation involved a factorial series of 16 talc dusts and in addition untreated and talc treated control groups.

The relative activity of the four chemicals was determined by a series of experiments with cuttings of seven species. The cuttings of six species were treated and planted in fresh builder's sand in greenhouse propagation frames (5, 8, 11, 14). *Thuja* cuttings were planted in a mixture of equal volumes of sand and peat humus (2). The details of the experiments are set forth in Table VI; the experimental design and treatment of data have already been described in Part 1 of this communication.

Results

Chemicals

Marked differences among the effects of chemicals are shown in Table VII. The relative order of activity varied for the different species of cuttings and for the types of response within species. It is apparent, on the average, that indolylbutyric and naphthylacetic acids were more active than either of the other two chemicals, which, for the most part, failed to differ significantly between themselves. However, potassium naphthylhexoate was beneficial to *Lonicera* cuttings but markedly injurious to those of *Weigela*. Naphthylbutyric was more effective than the hexoate in respect of root length of *Deutzia*. Indolylbutyric acid was better than naphthylacetic as judged by

TABLE VI

SPECIES, REPLICATES, AND DATES OF EXPERIMENTS

(10 cuttings to the group)

Species of cutting	Number of replicates	Date	
		Planted	Observed
Lonicera tatarica L.	3	Jan. 15, 1941	Feb. 6, 1941
Weigela florida (Sieb. & Zucc.) A.D.C.	3	Sept. 28, 1940	Jan. 20, 1941
Sambucus canadensis L.	4	Oct. 3, 1940	Dec. 9, 1940
Deutzia Lemoinei Lemoine	3	Sept. 30, 1940	Nov. 11, 1940
Iresine Lindeni Lem.	4	Oct. 6, 1940	Nov. 8, 1940
Berberis Thunbergii atropurpurea (D.C.) Chenault	4	Sept. 26, 1940	Dec. 27, 1940
Thuja occidentalis L.	3	Oct. 25, 1940	Feb. 2, 1941

number and length of roots of *Lonicera* and number of roots of *Weigela*. A reversed order of activity of these two chemicals was shown by data for the number of rooted cuttings and length of root of *Sambucus* and the number of dead *Berberis* and *Thuja* cuttings. It should be noted that rooting responses to these chemicals tended to vary inversely with number of cuttings showing green growth and the weight of green growth.

Concentrations

While the average effects of concentration are clearer than differences between chemicals they must be considered in relation to the plant species and the specific response. In general, the 4000 p.p.m. dosage was injurious, though this dosage favoured rooting of *Deutzia*, but the results failed to differ significantly from those at the 2000 p.p.m. concentration. In most respects the 1000 and 500 p.p.m. levels failed to differ significantly, though the 500 p.p.m. dosage produced fewer roots on *Deutzia* cuttings. The progressively disadvantageous effects of ascending concentration are clearly shown by data for the number of rooted and dead *Iresine* cuttings. Absence of significant average concentration effects for either *Lonicera* or *Weigela* for the number rooted is of particular interest since cuttings of the former species rooted in high percentages and of the latter in very low percentages. This lack of concentration effect at both extremes of the possible range of rooting was coupled with progressively receding injury with falling concentration for the other responses of these two species. This result suggested that percentage rooting alone is a relatively insensitive gauge of effectiveness of a range of concentrations of plant hormone chemicals.

Differential Responses to Chemicals and Concentrations

In only 4 of the 27 responses (the total considered for all seven species) were there significant differential responses to chemicals and concentrations.

TABLE VII

AVERAGE ROOTING RESPONSES OF PLANT CUTTINGS TO CHEMICALS AND DOSAGE

	Controls		Effects of chemicals averaged over the four dosages				Effects of dosage averaged over the four chemicals				Necessary difference, 5% level
	Untreated	Talc treated	Naphthyl-acetic acid	Naphthyl-butyric acid	Potassium salt of naphthyl-hexoic acid	Indolyl-butyric acid	Chemical concentration in talc, p.p.m. 4000	2000	1000	500	
Lonicera											
Number of cuttings rooted											
Transformed data	81.1	68.1	61.8	61.7	72.7	76.8					11.5
%	93	80	75	75	87	92					
Weight of new growth per cutting, gm.	0.52	0.46	0.44	0.51	0.58	0.46	0.43	0.50	0.53	0.53	0.08
Number of roots per rooted cutting	11.1	10.5	11.1	9.5	11.3	16.6					2.2
Length of root per rooted cutting, mm.	170	121	96	92	133	182	98	120	140	145	41
Mean root length, mm.	15.1	11.7	8.3	8.8	11.3	11.4	7.6	9.6	10.0	12.4	2.4
Weigela											
Number of cuttings rooted											
Transformed data	36.1	19.9	25.1	24.1	14.7	31.6					11.7
%	37	17	19	20	11	29					
Number of cuttings dead											
Transformed data	17.2	21.9					50.1	47.0	39.3	39.4	8.9
%	13	20					58	53	41	41	
Number of cuttings rooted or callused and with green growth											
Transformed data	63.9	68.1					29.1	39.3	47.7	46.8	9.1
%	80	80					29	41	54	52	5.3
Number of roots per group of 10 cuttings	11.3	1.7	4.8	5.7	1.8	10.5					
Length of roots per group of 10 cuttings, mm.	42	26	26	50	10	139					
Sambucus											
Number of cuttings rooted											
Transformed data	41.2	41.2	64.5	53.6	45.0	45.4	40.3	52.9	56.6	58.7	9.5
%	45	45	76	64	51	51	46	59	67	70	
Number of cuttings dead											
Transformed data	44.2	45.8	25.1	36.0	45.0	44.6	49.7	37.1	33.0	30.9	9.1
%	47	50	24	36	48	48	54	41	32	29	
Number of roots per rooted cuttings	10.6	4.8	14.9	7.3	8.4	16.8					3.3
Length of root per rooted cutting, mm.	176	79	234	120	109	214					21

TABLE VII—*Concluded*

AVERAGE ROOTING RESPONSES OF PLANT CUTTINGS TO CHEMICALS AND DOSAGE—*Concluded*

—	Controls		Effects of chemicals averaged over the four dosages				Effects of dosage averaged over the four chemicals				Necessary difference, 5% level
							Chemical concentration in talc, p.p.m.				
	Untreated	Talc treated	Naphthyl-acetic acid	Naphthyl-butyric acid	Potassium salt of naphthyl-hexoic acid	Indolyl-butyric acid	4000	2000	1000	500	
Deutzia											
Number of cuttings rooted											
Transformed data	44.7	43.1					70.4	67.7	59.5	60.1	8.4
%	50	47					85	83	73	74	
Number of cuttings with new growth											
Transformed data	77.7	81.1	60.7	80.3	74.4	69.6	54.9	71.4	78.9	79.9	11.9
%	93	93	71	92	88	83	62	86	92	92	
Number of roots per rooted cutting	3.7	5.4	13.0	9.6	9.0	11.3	13.5	11.7	10.3	7.5	2.2
Length of root per rooted cutting, mm.	26	17	26	26	20	28					4.8
Iresine											
Number of cuttings rooted											
Transformed data	78.8	85.4	48.4	70.5	66.2	48.5	38.4	54.1	69.7	71.4	10.5
%	92	97	57	84	78	56	42	63	84	85	
Number of cuttings dead											
Transformed data	11.2	4.6	36.0	19.5	23.8	41.5	46.0	35.9	20.4	18.6	10.6
%	8	3	37	16	22	44	51	37	16	15	
Number of roots per rooted cutting	20.6	18.0	44.7	34.1	29.0	47.2					10.1
Mean length of root mass, mm.	18.7	22.7	13.8	18.2	17.3	12.9					3.1
Berberis											
Number of cuttings dead											
Transformed data	85.5	72.0	87.8	67.7	75.9	78.6	85.1	80.1	74.8	69.9	7.2
%	98	88	98	82	89	92	96	92	89	84	
Thuja											
Number of cuttings dead											
Transformed data	15.0	19.9	64.5	48.6	51.7	47.5	68.2	59.9	43.5	40.8	13.2
%	10	17	74	57	60	53	82	70	47	45	

Differential effects on the number of roots per rooted *Lonicera* cutting are shown in Table VIII. The 4000 p.p.m. level of naphthylacetic and naphthylbutyric acids resulted in fewer roots than the 1000 and 500 p.p.m. levels suggesting that the highest dosage level was injurious. Potassium naphthylhexoate failed to show any significant difference over the entire concentration range. Marked increase in root number at the three higher levels was apparent for indolylbutyric acid.

TABLE VIII

DIFFERENTIAL EFFECTS OF CHEMICALS AND DOSAGE ON ROOTING OF CUTTINGS

Chemical	Chemical concentration in talc, p.p.m.			
	4000	2000	1000	500
Number of roots per rooted Lonicera cutting				
Naphthylacetic acid	8.1	10.4	14.2	10.6
Naphthylbutyric acid	7.4	8.2	9.9	12.5
Potassium naphthylhexoate	10.5	12.5	11.7	10.5
Indolylbutyric acid	21.3	15.9	17.5	11.8
Necessary difference, 5% level, 4.4				
Number of rooted Sambucus cuttings (transformed data)				
Naphthylacetic acid	51.0	78.7	57.8	70.5
Naphthylbutyric acid	55.5	45.0	53.2	60.8
Potassium naphthylhexoate	27.8	49.5	56.2	46.5
Indolylbutyric acid	27.0	32.2	59.2	57.0
Necessary difference, 5% level, 19.0				
Number of dead Iresine cuttings (transformed data)				
Naphthylacetic acid	39.9	55.3	27.7	21.2
Naphthylbutyric acid	28.2	28.8	6.7	14.4
Potassium naphthylhexoate	45.0	11.2	17.9	21.0
Indolylbutyric acid	71.0	48.1	29.1	17.9
Necessary difference, 5% level, 21.1				

It is also apparent from the data of Table VIII that the 4000 p.p.m. dosage of all chemicals but naphthylbutyric acid reduced rooting of *Sambucus* cuttings. Injurious effects of indolylbutyric acid were relatively more marked at the highest dosage than were those of naphthylacetic acid. Since mortality was inversely proportional to rooting it is unnecessary to give data to show that substantially similar differential effects were obtained.

Differential effects on the mortality of *Iresine* cuttings are shown in Table VIII. Indolylbutyric acid showed greater activity than the other chemicals at the 4000 p.p.m. level. Naphthylacetic and naphthylbutyric acids resulted in relatively low mortality at the two lowest dosages whereas potassium naphthylhexoate was markedly injurious only at the highest level.

Effects of Treatment with the Carrier Dust

Talc treated and untreated cuttings yielded results (Table VII) that tended to be similar. Untreated cuttings tended to have a slightly better response than those with talc treatment, particularly in relation to root length. However, talc treatment suggested beneficial effects as judged by the responses of *Iresine* cuttings and mortality of *Berberis*. Marked beneficial effects from talc treatment have been reported previously (11, 12, 14).

Discussion

Naphthylbutyric acid and potassium naphthylhexoate affected the rooting responses of plant cuttings but to a less marked degree than either naphthylacetic or indolylbutyric acids. Lack of activity cannot be attributed to a lower molecular concentration as was previously suggested (11), since the talc dusts described in this communication contained molecularly equivalent amounts of the chemicals. All four chemicals tended to show injurious effects at the 4000 p.p.m. dosage level.

The rooting responses of cuttings of seven plant species over the concentration range from 4000 to 500 p.p.m. have failed to demonstrate any particular merit for naphthylbutyric acid and potassium naphthylhexoate. While these chemicals can be used to stimulate rooting responses, plant propagators would be well advised to confine their practical interest to the more readily available naphthylacetic and indolylbutyric acids.

Results herein reported demonstrate the variability of chemical and concentration effects from species to species and for the varied responses within species. It is rather difficult to account for these differences. Obviously, applied dosage merely signifies available chemical potential. The permeability of the plant tissue to the chemical and its subsequent mobility and relation to the metabolism of the plant are probably limiting factors. It has recently been shown that phytohormone chemicals are effective dispersing agents for undenatured plant proteins (15). It may be possible that these chemicals affect the rooting responses of plant cuttings through their effects on the protein system. Should this suggested mechanism be correct, fundamental differences in the plant protein from species to species might account in part for variability in the effects of various chemicals.

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A RAPID SQUASH TECHNIQUE FOR STEM AND ROOT TIPS[1]

By L. P. V. Johnson[2]

Abstract

A method of preparing permanent slides of stem and root tips for chromosomal studies is described. It involves the following procedure:

1. Fix in Carnoy's fluid, 30 min.
2. Macerate and hydrolyse at room temperature in a mixture of concentrated hydrochloric acid and 95% ethanol (1 : 1) 5 to 10 min.
3. Rinse as quickly as possible in two changes of water.
4. Stain in leuco basic fuchsin, one hour (or longer).
5. Transfer material to drop of 50% acetic acid on slide, cover, squash by tapping with a pencil, and, protecting cover glass with absorbent paper, apply pressure with finger. Allow to stand until drying begins under cover glass (about 10 min.).
6. Remove cover glass, flood both cover and slide twice with 95% ethanol and three times with *n*-propyl acetate at about half-minute intervals, place drop of Canada-balsam–xylene on slide and replace cover glass.

The method has proved satisfactory in over 20 species, mainly in Pinaceae.

Introduction

In studying the effect of colchicine treatment on seedlings of some 20 forest tree species, it was necessary to make chromosome counts on several hundred plants. For the most part these counts were made from preparations of the growing points of the stem, since the colchicine treatment was commonly applied to that part of the plant. Counts from root tips were also extensively made.

The extent to which routine chromosome counting was involved in this work provided opportunities for testing existing 'squash' and 'smear' methods, such as those of Hillary (5) and of Warmke (8), and also for attempting improvements on them, especially with regard to shortening of the procedures. The present work represents the advantage taken of these opportunities.

Materials and General Methods

The species used in these studies were: *Abies grandis*, *Allium Cepa*, *Arachis hypogaea*, *Betula lutea*, *Carthamus tinctorius*, *Lycopersicum esculentum*, *Picea Abies*, *P. glauca*, *P. mariana*, *P. sitchensis*, *Pinus Banksiana*, *P. contorta latifolia*, *P. koraiensis*, *P. monticola*, *P. ponderosa*, *P. resinosa*, *P. rigida*, *P. Strobus*, *P. sylvestris*, *Populus alba*, *P. canescens*, *P. grandidentata*, *Pseudotsuga taxifolia*, *Spinacia oleracea*, *Thuja plicata*, and *Tsuga heterophylla*.

In 1942-43, in order to stimulate cell division in stem tips of greenhouse materials, potted plants were placed for several hours under intensified condi-

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tions of light and temperature, controlled by a system of light bulbs, reflectors, and ventilators. This procedure showed definite promise in early winter, but as the normal greenhouse growth increased in late winter an abundance of dividing cells was obtained in preparations from untreated plants, obviating any need of forcing. In later years the practice had been to wait for this flush growth to appear.

The colchicine pretreatment method of Meyer (6) was also tested in the greenhouse by immersing detached stem tips in 0.2% colchicine for one or two hours in light. This pretreatment caused shortening and straightening of the chromosomes and, through prevention of spindle formation, permitted distribution of the compact chromosomes throughout the flattened cell. It was used considerably in coniferous species, the chromosomes of which are unusually long.

Cytological observations were commenced in December, 1942, and have been continued intermittently since that time on both greenhouse and field materials.

Experimentation on methods led from the testing of smear and squash techniques of other authors (5, 8) to the development of modifications, especially in shortening the procedure. The work was then resolved into the testing, in various combinations, of fixing agents (Carnoy's—with modifications, ethanol–acetic-acid, La Cour's 2 BE, and Bouin's), macerating agents (4% sodium hydroxide at 60° C., various concentrations and combinations of hydrochloric acid, ethanol, and water, and of hydrochloric acid, acetone, and water), stains (leuco basic fuchsin, iron-aceto-carmine, toluidine blue, methylene blue, chlorazol black E, and crystal violet), and dehydrating agents (1, 4-dioxane, absolute ethanol, isopropyl ether, isopropyl acetate, *n*-propyl acetate, and acetone). These experiments led to the testing of nearly 50 distinct procedures, the superior one of which (No. 38) is outlined in the present paper.

Method 38

In January, 1943, after making nearly 300 permanent slides by previous methods, Method 38 was evolved. This method was tested on both stem and root tips of the entire range of materials listed in the previous section. While proving generally satisfactory, it was especially good for the coniferous species, in which materials it left little to be desired. For tender material, such as tomato root tips, a reduction in the strength of the macerating agent (down to equal proportions of 95% ethanol and 25% hydrochloric acid) was found beneficial.

Over 100 slides prepared by Method 38 remain in excellent condition after 15 months.

The method is outlined below after the style being used in *Staining Procedures* (4):

Specially recommended for: stem and root tips, especially in Pinaceae.

Designed to: provide a rapid squash method for chromosomal observations.

Preparation of leuco basic fuchsin:

Dissolve 1.0 gm. basic fuchsin by pouring 200 ml. boiling distilled water over it. Shake thoroughly, cool to 50° C., and filter. Add 2.0 gm. potassium metabisulphite and 20 ml. normal hydrochloric acid to the filtrate, shake, stopper, and bleach 24 hr. in the dark. Add 0.5 gm. decolorizing carbon, shake one minute, filter through coarse filter paper. (After de Tomasi (7) and Coleman (2))

Schedule:

1. Fix in Carnoy's fluid, 30 min.
2. Macerate and hydrolyse at room temperature in a mixture of concentrated hydrochloric acid and 95% ethanol (1 : 1) 5 to 10 min.
3. Rinse as quickly as possible in two changes of water.
4. Stain in leuco basic fuchsin, one hour (or longer).
5. Transfer material to drop of 50% acetic acid on slide, cover, squash by tapping with a pencil, and, protecting cover glass with absorbent paper, apply pressure with finger. Allow to stand until drying begins under cover glass (about 10 min.).
6. Remove cover glass, flood both cover and slide twice with 95% ethanol and three times with *n*-propyl acetate at about half-minute intervals, place drop of Canada-balsam–xylene on slide and replace cover glass.

Notes on schedule:

Stem tips are trimmed so that only youngest leaves and meristematic tissue remain. Material is then placed in Carnoy's fluid contained in 5 to 10 ml. shell vial held upright in holder. Material is not removed from vial until it is transferred to the slide in Step 5. It is usually advantageous further to trim material in Step 5 (in drop of 50% acetic acid at one end of slide) since stain discloses actively dividing tissue. Then move trimmed material to another drop at centre of slide. In Step 6, Kalteyer's forceps are used to hold cover glass when flooding.

Discussion

Method 38 has, so far as is known, the advantage of being the quickest and simplest procedure of its kind yet devised, and is satisfactory, for superficial chromosomal observations at least, for a wide range of material.

The only strictly new feature is the use of *n*-propyl acetate as a dehydrating agent. The advantage of this chemical over 1, 4-dioxane (found to be the best of the standard dehydrating agents) is, so far as the present studies are concerned, a matter of relative toxicities. There have been several reports on the toxic effects of dioxane vapour (1, 3) some of which record human fatalities. On the other hand, *n*-propyl acetate has been shown to have low toxicity and, although used in the lacquer and varnish industry, no toxic effects have been reported in man (1). The author has used it extensively for cytological dehydration for over two years without special precautions and without ill effects.

It was found that the use of 95% ethanol prior to *n*-propyl acetate shortened the total time required for dehydration. This is probably related to the greater penetration and water-solvent properties of ethanol, which enabled it better to act on the relatively high initial water content of the tissue. The role of *n*-propyl acetate, in which water dissolves 1.89% at 20° C., was then to remove the slightly diluted ethanol and to complete the dehydration of the relatively water-free tissue.

An important property of *n*-propyl acetate is that it does not take up water from the air, which gives it a very definite advantage over absolute ethanol.

Acknowledgments

The author is grateful to Dr. D. F. Stedman for advice respecting dehydrating chemicals, to Dr. C. Heimburger for testing Method 38, and to Mr. H. W. Holtz for his interest and assistance in the greenhouse phases of the work.

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TETRAPLOID *TARAXACUM KOK-SAGHYZ*

I. CHARACTERS OF THE LEAVES AND INFLORESCENCES IN THE PARENTAL COLCHICINE-INDUCED GENERATION[1]

By M. W. Bannan[2]

Abstract

Seedlings at different stages of development were treated with colchicine. Successive selections on the aspect of the seedlings soon after treatment, on size of the guard cells at the time of repotting, and finally, after overwintering, on counts of the chromosomes in the pollen mother cells yielded a few hundred plants with tetraploid crowns. These plants bore fewer, broader leaves and fewer, bigger inflorescences with larger achenes than did selected large-celled diploids given the same treatment, but in general the plants were no bigger. A few of the tetraploids were self-fertile (if not apomictic) early in the spring, but later all tested plants proved self-sterile.

Introduction

Among the various rubber-producing plants *Taraxacum kok-saghyz* Rodin ranks as one of potential importance, but the plants are small and field cultivation from seed is difficult. Accordingly, attempts have been made to solve the problems of culture, improve the rubber content, and increase the size of roots. Production of tetraploid kok-saghyz by the colchicine technique was first reported by Kostoff and Tiber (9), followed shortly by Navashin and Gerassimova (12, 13). According to these authors, and descriptions given by Nichiporovich, Ostapenko, and Vassiljeva (14), the polyploid plants had larger roots, inflorescences, and achenes than the diploids. Recently Warmke (23) has also reported bigger roots among the tetraploids.

The objects of the present investigations were to produce tetraploid plants and, if these gave evidence of improvement over diploid kok-saghyz, to select and propagate the most promising types. In order to obtain polyploids the colchicine method was used. However, because of the possible effects of colchicine on growth, quite apart from the cytological changes induced by the drug, and also because most of the treated plants become chimaeras, it was considered that the critical determination of the effects of tetraploidy should be made from second generation plants. Consequently the cytological analyses of the colchicine-treated plants were made from the reproductive parts rather than the root tips, and those inflorescences that were determined as tetraploid were cross-pollinated for the obtaining of polyploid seed. The present paper deals with certain aspects of the production of the first generation plants and the characteristics of their leaves and inflorescences.

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Methods

In the initial experiments early colchicine treatments were attempted and germinating seeds were immersed in colchicine solutions of various strengths two days after the application of water. Germination was highly uneven and the stage of advancement in the two-day materials varied from that of a protruded radicle several millimetres in length to one of no external evidence of germination. Manifestly the individuals in each lot were at different stages of development when treated with colchicine. It presently became clear that the less advanced specimens were being treated too early. Therefore in subsequent experiments the seedlings were sorted as they germinated and successive selections were made for each treatment with colchicine. Thus all the individuals in each lot were at a similar stage of development when colchicine was applied. A total of 142 lots comprising about 27,000 individuals were thus treated. The age of the seedlings treated ranged from 36 hr. to 47 days from the initial soaking in water. The concentration of colchicine solutions varied from 0.0025 to 0.40% and the duration of immersion from 1.5 to 24 hr. After removal of the colchicine the seedlings were washed in several changes of water and planted in flats.

Differences soon became apparent among the seedlings in the flats. Some appeared to have been unaffected by the colchicine, since they grew normally and resembled the untreated controls. These were discarded as probable diploids. Other seedlings were profoundly altered. Some of these soon perished, while others grew slowly and produced much deformed leaves exhibiting such abnormalities as asymmetry, irregularly cleft margins, and rugose surfaces. Plants of this type were considered too heterogeneous cytologically to prove valuable and the majority were discarded. Yet other seedlings were initially retarded but on resumption of growth developed leaves that were thicker than usual but not otherwise deformed. These specimens were selected as possible tetraploids and were planted in 2-in. pots. All together some 2700 such seedlings were selected, or about 1 in 10 of the total number treated with colchicine.

When the rosettes attained a diameter of 2 to 4 in. selected plants were transferred to large pots, either singly in 7- or 8-in. pots or two together in 9-in. pots. Since it was impracticable to repot all 2700 plants, a second selection was made at this time. Numerous authors (Babcock and Stebbins (1), Blakeslee (3), Franco (6), Randolph (16), Sax and Sax (19), Sax (20), and Smith (22)) have shown that the stomata are usually larger in polyploids than diploids, and that size of the stomata can be utilized for provisional sorting of such plants. Since there was insufficient time for cytological examination of root tips, stomatal dimensions were used instead. Examination of untreated diploids growing in garden plots revealed the guard cells to be generally 25 to 30μ in length, and accordingly it was decided to take a length of 35μ as a minimum for possible polyploids. Of the 2700 plants checked at repotting 935 possessed guard cells of this or greater size and hence were selected as potential polyploids.

After the second selection and repotting, the plants were divided into groups and given different overwinter treatments as follows.

Series *A*. After repotting in October, 1943, the plants were placed in a temperate greenhouse. From Dec. 10 to Feb. 8, 1944, they were in a cool house heated only by convection through an open door, the temperatures ranging from 30° F. at night to 70° F. on sunny afternoons. On Feb. 8 the plants were moved into an unheated cold house. Here the temperature sank below 32° F. on 13 nights during the remainder of the month, reaching a minimum of 19° F., and exceeded 70° F. on bright afternoons. No freezing temperatures were experienced during March and toward the end of the month growth was resumed.

Series *B*. Plants of this series were given the same chilling as Series *A*, but were 1 to 2 months younger, having been repotted in November and December.

Series *C*. The plants were repotted in October and November, 1943. From Dec. 10 to Feb. 8 they were in the cold house where they experienced temperatures below 32° F. on seven occasions. On Feb. 8 they were moved into the cool house where the temperatures during the remainder of the month fluctuated between 40° F. and 80° F. These plants were the first to resume growth, the earliest leaves appearing during the latter part of February.

Series *D*. These plants were the oldest, having been transferred to large pots in September, 1943. They resided in the temperate house until late October or early November when they were moved in two lots to cold frames outside. At this time a few were in flower. In early December the plants were covered with excelsior and loose sash laid across the tops of the frames. Of the various series this was the last to resume growth in the spring.

Series *E*. This series was made up of a few of the youngest plants that were repotted in December, 1943. They were kept in a heated house until removal to the cool house in February. During April they were moved outside, but at no time were they exposed to temperatures below the freezing point.

The plants of Series *A*, *B*, and *C* were kept in the greenhouses during the spring. They were watered every day, and at intervals nutrient solution was given in addition. After the cessation of flowering in July the pots were sunk outside. The plants in Series *D* received less attention. They were watered irregularly, and after removal from the cold frames in June the pots were left in the open for approximately one month before being sunk.

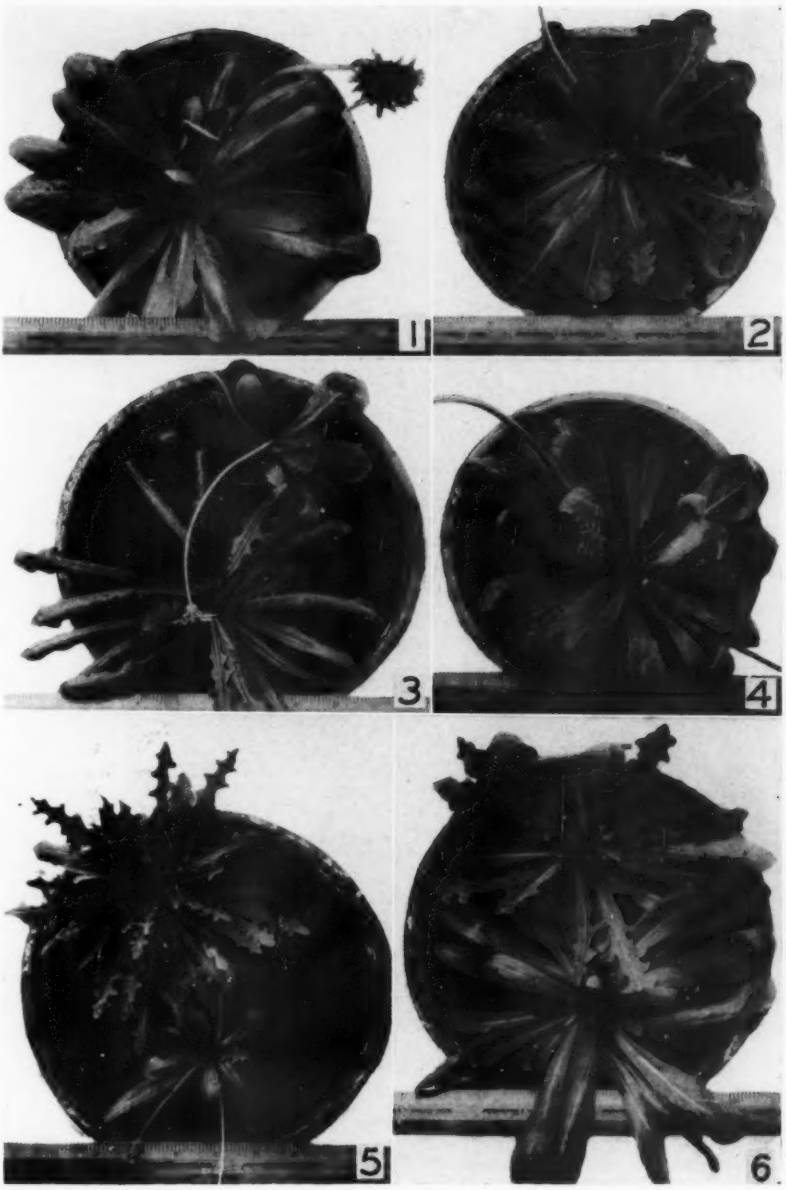
As the first flower buds appeared in the spring, chromosome counts were made from the pollen mother cells, and whenever possible these were checked by examination of later formed heads. Plants with the most promising appearance were examined first and since little time remained after the determination of these, only a few of the plants exhibiting minor abnormalities were checked. In this connection it might be recalled that the most deformed plants, numbering several thousand, had previously been discarded as hopeless mixoploids. Of the 600 specimens examined, 387 produced tetraploid

heads and 153 diploid heads. Others showed cytological abnormalities of different types or were recognized as chimaeras. Some of the latter were sectorial, with diploid heads on one side and tetraploid on the other, or less often some heads were tetraploid and others octoploid. In the case of the few deformed individuals that were examined various numbers of chromosomes extending up to octoploidy were observed.

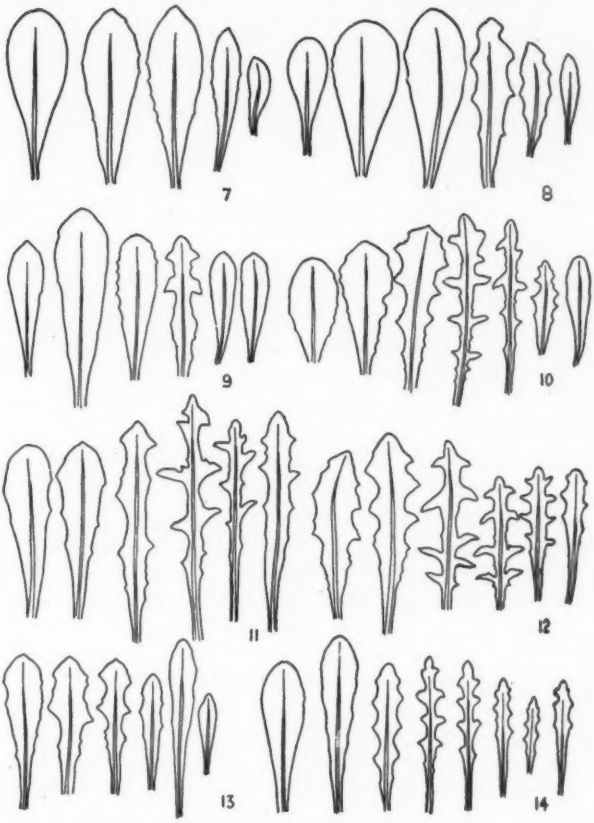
Leaves

Among the colchicine-treated plants were some that stood out from the others because of the spatulate or broadly oblanceolate shape of the first-formed leaves (Figs. 1, 2, 3 (upper), and 4). These were generally revealed to have tetraploid heads, so that there came a tendency to associate this shape with tetraploidy and to attempt to identify plants among the population as diploid or tetraploid according to their leaf shape. However, it soon became apparent that this criterion was not infallible. Sporadic plants with broad leaves produced heads with diploid pollen mother cells. Conversely, although those plants that had lanceolate leaves from the beginning usually proved to be diploid (Fig. 6), some turned out to have tetraploid heads (Fig. 5). In the case of three recognized sectorial chimaeras, the leaves on the diploid and tetraploid sides looked alike and only careful comparison revealed them to be slightly wider on the tetraploid side. The width of the spring and early summer leaves of representative diploids and tetraploids was not measured, but the width appeared slightly greater in the tetraploids. Some measurements were made of the narrower leaves developed in late summer, and here the width was 20% greater among the tetraploids. Thus it may be stated that the leaves were generally slightly wider in the plants with tetraploid heads than in those with diploid heads, but the variation was so great in both that neither could be identified with certainty on the basis of leaf shape. Possibly some of the variants were unrecognized chimaeras.

The nature of the leaf margin was even more variable than leaf shape, and no consistent relationship with chromosome number was observed. Figs. 7 to 12 illustrate the range in type of leaf margin among tetraploids. In some plants, as shown in Fig. 7, the early spring leaves were entire, the summer leaves only shallowly and sparsely indented, and the autumn leaves again entire like the spring leaves. In contrast, other plants possessed rosettes in which even the first-formed leaves were deeply cut (Fig. 12). Between these extreme types were found the majority of plants, the initial leaves of which were more or less entire, while the summer leaves possessed margins cut to varying depths (Figs. 8 to 11). In all cases the leaves formed during rejuvenescence tended to revert to the spring type. The increasing dissection of the summer leaves and the reversion of the autumn leaves to the juvenile spring type have been described by Sears (21) as a general characteristic of *Taraxacum*. An equally wide range in character of the leaf margin was noted in the treated diploids. Some plants had entire leaf margins (Fig. 6, lower),



FIGS. 1 to 6. *Colchicine-treated diploid and tetraploid Taraxacum kok-saghyz;* 1, 2, 3 *(upper)*, 4, and 5 *(both)—tetraploid;* 3 *(lower)* and 6 *(both)—diploid.*



FIGS. 7 to 14. *Seasonal alterations in leaf size, shape, and margin in Taraxacum kok-saghyz;* 7 to 12, *tetraploid plants;* 13 and 14, *selected treated diploid plants.*

while others had very deeply cut leaves. Figs. 13 and 14 illustrate the more common intermediate condition.

The length of the leaves was likewise variable, and again no consistent differences were noted between diploids and tetraploids. Among colchicine-treated plants, the range of variation in the maximum diameter of the rosette was greatest in diploids, varying from 4 to 20 in., but, as shown in Table I, the averages for diploids and tetraploids were similar. This is apparent in each of the four series. Contrasting the various series, it will be seen that the largest size was attained by plants in Series *A* and *B*, which had both a shorter period of exposure to cold and more favourable growing conditions during the spring than Series *D*. Plants of the latter series overwintered in the cold frames and were the last to resume growth in the spring and also to exhibit rejuvenescence after summer dormancy. When the rosettes of Series *D* were

TABLE I

DIAMETER OF ROSETTES OF TETRAPLOIDS AND SELECTED TREATED DIPLOIDS

Series	Type	Number of plants	Range and average diameter in in. (1944)			
			Apr.	June	July	Sept.
A	2*n*	11	—	7-20 (11.2)	6-14 (9.1)	2-8 (5.5)
	4*n*	42	—	6-15 (10.9)	6-13 (8.6)	1-8 (3.9)
B	2*n*	10	—	8-16 (11.2)	6-13 (10.5)	6-9 (6.9)
	4*n*	32	—	4-14 (11.6)	5-13 (9.4)	1-9 (4.6)
C	2*n*	31	4-15 (9.4)	3-13 (8.8)	3-12 (7.0)	2-10 (5.5)
	4*n*	136	5-14 (9.4)	5-14 (8.9)	1-11 (6.5)	1-9 (5.3)
D	2*n*	45	—	—	4-11 (7.8)	0-7 (2.6)
	4*n*	87	—	—	3-12 (7.7)	0-8 (3.0)

enlarging during June and July, those of Series *C*, the first to grow in the spring, were diminishing at the onset of summer dormancy.

The diploids and plants with tetraploid crowns among the colchicine-treated population differed strikingly in the production of leaves. Although the maximum number of leaves in the rosette at one time was highly variable, ranging from 12 to 171 in the plants studied, the diploids on the average had 40 to 70% more leaves than the tetraploids. As shown in Table II this degree of difference was apparent in each of the four series. The data also show the earlier rejuvenescence of the diploids.

TABLE II

NUMBER OF LEAVES IN ROSETTES OF TETRAPLOIDS AND SELECTED TREATED DIPLOIDS

Series	Type	Number of plants	Range and average number of leaves (1944)			
			May	June	July	Sept.
A	2*n*	11	—	39-143 (71)	11-104 (34)	26-171 (66)
	4*n*	41	—	13-77 (41)	8-46 (21)	1-60 (21)
B	2*n*	11	—	22-75 (44)	17-54 (32)	11-135 (67)
	4*n*	32	—	15-67 (29)	6-55 (21)	2-82 (25)
C	2*n*	29	13-70 (37)	7-82 (35)	3-58 (27)	14-105 (46)
	4*n*	136	8-84 (26)	6-82 (24)	2-61 (18)	3-103 (29)
D	2*n*	45	—	—	14-82 (30)	0-40 (13)
	4*n*	87	—	—	8-42 (21)	0-47 (10)

Comparative studies of the epidermis were made of a considerable number of diploids and tetraploids. These consisted of plants selected at random from the colchicine-induced tetraploids, treated plants that remained diploid, and untreated second-year diploids, both potted and from the field. Although

size and shape of the epidermal and guard cells varied considerably there were on the whole noteworthy differences between the tetraploids and most diploids. Figs. 17 and 19 give some indication of the range of cell size in diploids, the size in Fig. 19 approximating the minimum in diploids generally, while that in Fig. 17 is somewhat above the average for selected treated specimens. For comparison with tetraploids reference may be made to Figs. 18 and 20. Fig. 18 depicts the average size of cells observed in tetraploids and Fig. 20 the maximum. Contrasting Figs. 18 and 20 it may be noted that the guard cells are definitely larger in Fig. 20 but the epidermal cells not noticeably so.

Variations in size of guard cells and frequency of the stomata correlated with environmental conditions have been reported for several genera by Isanogle (8), Penfound (15), and Reed and Hirano (17). Similar variations were observed in kok-saghyz. Ontogenetic trends were also evident. As a rule the broad spring leaves had larger and more widely spaced stomata than the narrower summer leaves. For example, the average number of stomata per sq. mm. in the selected treated diploids increased from 77 for early spring leaves to 80 for late spring leaves and to 95 for summer leaves. The corresponding frequencies in tetraploids were 54, 69, and 84 stomata per sq. mm. Sometimes the frequency remained relatively constant among leaves of similar age, but in other cases considerable fluctuation occurred. There was also variation in the size and distribution of stomata in different parts of the same leaf. Long and Clements (10) demonstrated such variation in *Chrysanthemum maximum* some time ago.

In order to obtain as accurate a comparison as possible of the stomata in diploids and tetraploids, all measurements were made from similar parts of leaves produced at like stages in the development of the plant. The determinations were made from the distal portions of fully grown leaves formed when the rosettes were at the peak of development. This stage was attained in June by Series *A* and *B* and through July by Series *D* and the untreated diploids growing in the field. All plants were in the second year of growth. The guard cells of 40 stomata were measured and, with the aid of a grid, counts were obtained of the number of stomata in the low power field having an area slightly under 2 sq. mm. Two or more determinations were made from different areas, avoiding the largest veins. The data are presented in Figs. 15 and 16.

The stomata in the plants with tetraploid crowns were large and widely spaced, the mode being 50 to 59 stomata per sq. mm. as contrasted with 100 to 109 stomata per sq. mm. in untreated diploids. As expected, the size and frequency of stomata in the selected treated diploids more or less paralleled that in the tetraploids. These diploids were selected at repotting as possible tetraploids because they had large stomata, and in the matter of cell size they probably represent the upper portion of the diploid range. There were also indications that certain of these plants were not totally diploid but had an admixture of tetraploid tissue. It should be emphasized that the histograms for colchicined diploids in Figs. 15 and 16 do not present a complete

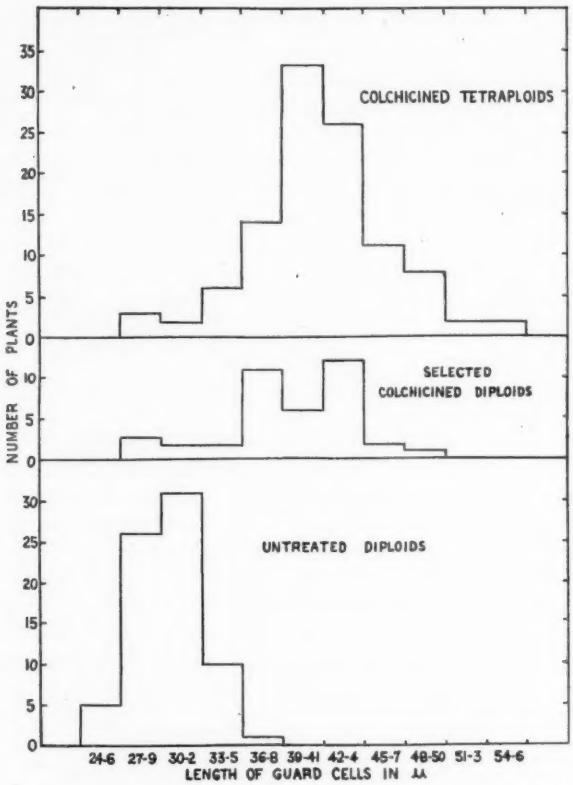


FIG. 15. *Length of guard cells in tetraploids, selected treated diploids, and untreated diploids.*

picture of stomata size and frequency in all treated diploids, but rather only in those selected as possible tetraploids. The small-celled individuals, comprising two-thirds of the 2700 plants examined at repotting, were discarded.

In summarizing the observations on leaves, it may be stated that the plants with tetraploid heads had definitely fewer but somewhat broader leaves than selected large-celled diploids. Leaf shape and margin were highly variable. Stomata were considerably larger in tetraploids than in all but the largest celled of the diploids.

Inflorescences

Total head production of the selected diploids and tetraploids during the summer of 1944, the second year of growth, is given in Table III. It will be observed that in each series the diploids produced twice as many heads as the plants with tetraploid crowns. Noteworthy differences also appeared between the various series. The largest number of heads was produced by

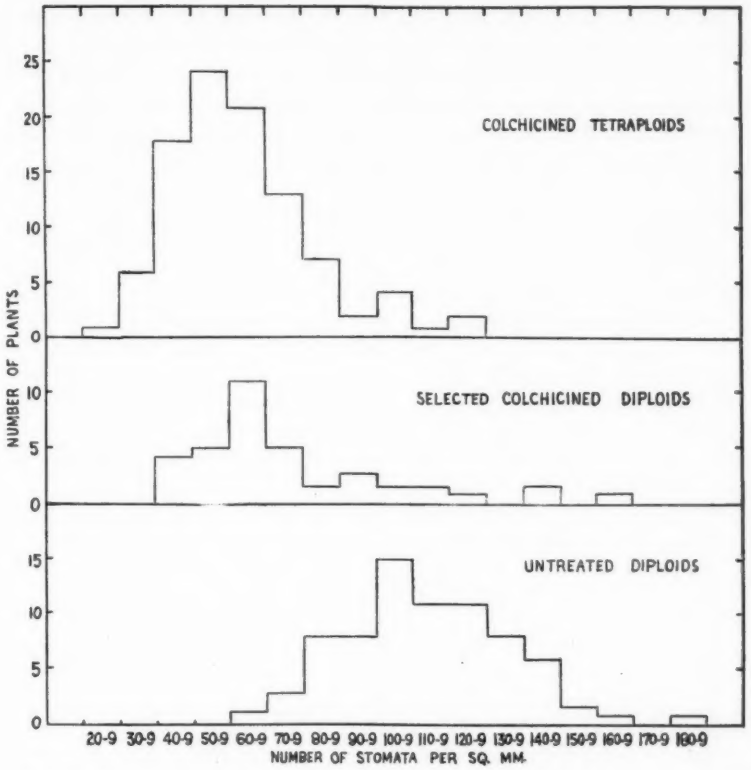

COLCHICINED TETRAPLOIDS
SELECTED COLCHICINED DIPLOIDS
UNTREATED DIPLOIDS
NUMBER OF PLANTS
NUMBER OF STOMATA PER SQ. MM.
20-9 30-9 40-9 50-9 60-9 70-9 80-9 90-9 100-9 110-9 120-9 130-9 140-9 150-9 160-9 170-9 180-9

FIG. 16. *Frequency of stomata in tetraploids, selected treated diploids, and untreated diploids.*

TABLE III

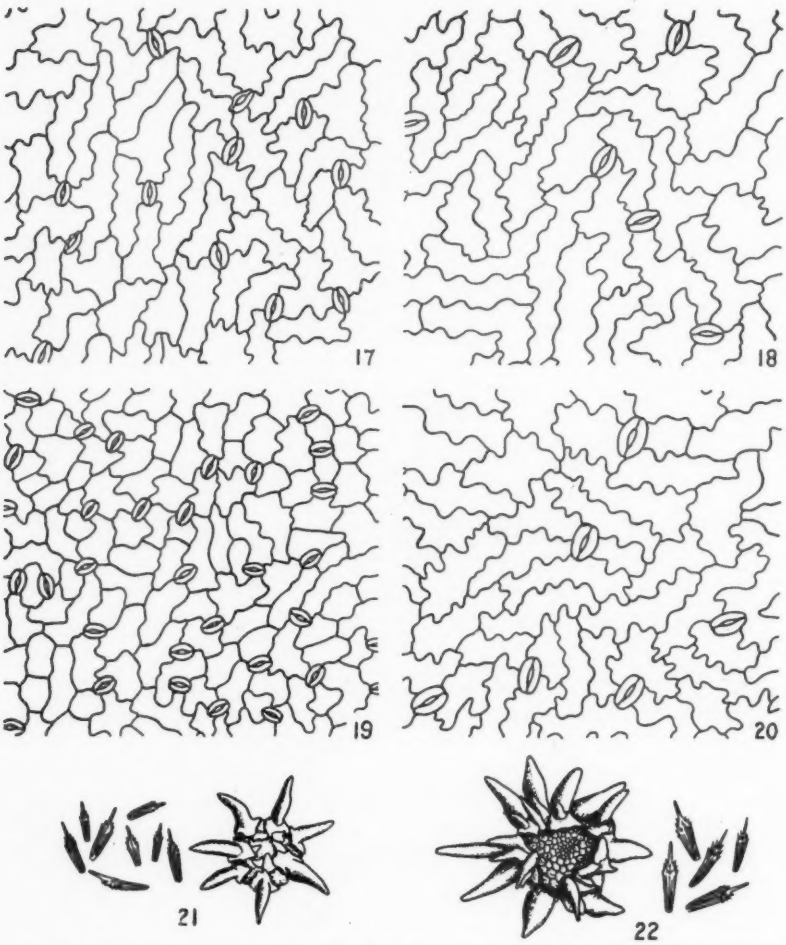
NUMBER OF HEADS PRODUCED DURING SECOND YEAR BY TETRAPLOIDS AND SELECTED TREATED DIPLOIDS

Series	Number of plants		Range and average number of heads	
	2*n*	4*n*	2*n*	4*n*
A	15	45	8-98 (43)	4-57 (22)
B	15	52	4-64 (28)	2-28 (11)
C	63	180	2-118 (24)	2-53 (14)
D	54	96	1-47 (21)	1-45 (12)
E	3	5	5-17 (10)	3-11 (7)

the plants in Series *A*, fewer were found in Series *B*, *C*, and *D* (the number decreasing in that order), and very few were borne by the plants in Series *E*. No counts were made of the heads produced by some untreated diploids of

only slightly greater age growing in pots, but flowering was profuse, apparently greater than in either colchicined diploids or tetraploids.

The differences in size of diploid and tetraploid heads were well illustrated by two sectorial chimaeras. Representative heads from one of these plants are depicted in Figs. 21 and 22. The tetraploid heads were about 50% greater in diameter than diploid heads on the same plant, they had larger and



FIGS. 17 to 22. *Lower epidermis, flower heads, and achenes of colchicine-treated diploid and tetraploid Taraxacum kok-saghyz.* 17 and 19. *Different portions of same leaf of diploid plant.* 18. *Average size of stomata in tetraploid.* 20. *Maximum size of stomata in tetraploid.* 21. *Diploid flower head of sectorial chimaera and random diploid achenes.* 22. *Tetraploid head from same chimaera and random achenes.*

more numerous involucral bracts bearing more prominent projections, and the peduncles were longer and thicker. The average number of florets per tetraploid head was 86, as contrasted with 52 per diploid head.

As a rule similar differences were noted between diploid and tetraploid plants, although much variation occurred in both. Counts were made of the florets in 330 heads from 56 tetraploid plants and 40 heads from seven colchicined diploid plants. The range was great, fluctuating from 45 to 152 florets per head for the tetraploids and 43 to 101 for the diploids, but the averages were quite distinct, being 94 for the tetraploids and 75 for the diploids. Here it might be noted that the number of florets varied with the time of flowering. As a rule the first heads had fewer florets, the number increased to a peak when head production was at a maximum, and fell off again in the last heads.

Many of the tetraploids were tested for self-sterility. For selfing two procedures were followed. Some heads were bagged individually and vigorously roughed with a sterile needle three or four times during each of the two successive days when the heads were open. In other cases, particularly when several heads on the same plant opened simultaneously, heads were rubbed together. Such heads were sometimes bagged individually, sometimes two together in a single bag. A total of 644 heads belonging to 169 plants were tested. Of these plants 150 were self-sterile, either failing to set any seed whatsoever or producing achenes from less than 1% of the florets. Nine plants were either slightly self-fertile or apomictic, producing 1 to 5% achenes. Ten other plants exhibited a 6 to 36% production of apparently good achenes. Some 40-odd of these achenes were tested for germination. Without special treatment nearly half germinated, and the root tips of the two seedlings checked cytologically were tetraploid. It should be noted that practically all the plants that were self-fertile (if not apomictic) belonged to Series *C*. Plants of this series were the first to bloom, flowering being at its peak in late May and early June. Little or no self-fertility was observed in Series *A* and *B*, which came into bloom about three weeks later.

Nearly a thousand heads of tetraploid plants were cross-pollinated and as a rule very broad cross-fertility was indicated. For instance, one plant appeared to be fertile in all crosses with 10 other plants. Occasional plants were highly sterile because of abortive pollen or other miscarriages of the reproductive processes.

The tetraploid achenes were noticeably larger than the diploid ones produced at the same time by plants of similar age (Figs. 21 and 22). A comparison of several hundred achenes revealed the weights of the achenes from tetraploids, large-celled treated diploids, and diploids growing in garden plots to be 0.66, 0.51, and 0.38 mgm. respectively.

Germination tests on simultaneous collections from colchicine-treated diploids and tetraploids gave the results in Table IV. Germination of the tetraploid achenes tended to lag slightly behind that of the diploid, but there was no noteworthy difference in total germination after a few days. Some

root tips were examined and the chromosome counts confirmed. The seedlings were set out in flats and during the initial stages the diploids in some lots grew slightly faster than the tetraploids, while in other cases growth rates were similar.

TABLE IV

GERMINATION TESTS OF UNTREATED ACHENES

Age of achenes, months	Germination after five days' soaking	
	2*n*	4*n*
1	0	0
2	60% (50 achenes)	60% (50 achenes)
3	77% (195 achenes)	73% (384 achenes)

Discussion

The work of many investigators (Blakeslee (4), Bain and Dermen (2), Dermen and Bain (5), Graner (7), Mendes (11), Satina, Blakeslee, and Avery (18), and others) has shown that a large proportion of the plants treated with colchicine become chimaeras. This was also evident in kok-saghyz where most of the seedlings became grossly deformed and were discarded as unpromising mixoploids. Of the many thousands of seedlings treated only a few hundred plants were obtained with tetraploid or predominantly tetraploid crowns, and indications are that perhaps the majority of these had diploid roots. Hence it is clear that decisions on the effect of tetraploidy, especially on the roots, must rest until information is provided by clean second generation plants grown from polyploid seed.

Regarding the aerial portions of the plants, it may be stated that the plants with tetraploid (or predominantly tetraploid) crowns were no bigger on the average than the selected large-celled plants given similar treatment but which remained diploid or retained a predominance of diploid tissue. The individual parts of the plants with tetraploid crowns tended, however, to be of greater size. The leaves and inflorescences were bigger, the compensating circumstance being that there were fewer of them. The situation may be similar to that reported for maize by Randolph (16) who stated that the tetraploid plants were no bigger than the diploid, but did have larger organs such as leaves, tassels, ears, and kernels.

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